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The Missoula Poplar Project: Utilizing Poplars to Enhance Wastewater Treatment

Heath Nicolas Carey
The University of Montana

THE MISSOULA POPLAR PROJECT:

UTILIZING POPLARS TO ENHANCE WASTEWATER TREATMENT

By

Heath Nicolas Carey

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Approved by:

Perry Brown, Associate Provost for Graduate Education
Graduate School

Cory C. Cleveland, Chair
Forestry

Steve W. Running
Forestry

Garon C. Smith
Chemistry

Redefining Dilution: An Alternative to Traditional Wastewater Treatment

Chairperson: Cory C. Cleveland

Wastewater treatment plants rank second to agricultural runoff in the top ten major pollution sources to U.S. surface waters. Such nutrient-rich inputs can degrade aquatic ecosystems by accelerating eutrophication events, especially in summer months when surface water flows are low. Alternative treatment practices, modeled after natural ecosystem processes, could reduce nutrient inputs to surface waters while accumulating biomass and sequestering atmospheric carbon dioxide. I designed and implemented an alternative treatment strategy, using effluent to fertilize trees at the Missoula Wastewater Treatment Facility. The objectives of this work were to assess: 1) environmental impacts of effluent application; 2) tree survivorship; and 3) growth effects. A two acre plantation was established in May 2009 by planting 316 dormant, unrooted stem cuttings of two hybrid poplar species, *Populus deltoides X Populus trichocarpa* and *Populus deltoides X Populus nigra*, and the native Black Cottonwood, *Populus balsamifera* ssp. *trichocarpa.* The effects of effluent fertilization on poplar growth, soil and ground water nutrient contents were monitored throughout the first growing season of this pilot project. Effluent fertilization nearly doubled poplar growth, and as suspected, had no major impacts on soil or ground water nutrient concentrations. Continued research at this site is necessary to observe environmental impacts as effluent loading rates increase. Our initial results suggest that surface application of wastewater effluent offers a valuable strategy for decreasing effluent input rates to the Clark Fork River. Moreover, this project offers smaller communities a "blue print" from which to design similar projects that remediate nutrient-rich effluent in a cost-effective way.

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Introduction

Nitrogen [N] is a vital component necessary for life on Earth. It ranks fourth behind oxygen, carbon and hydrogen as the most common chemical element in living tissues (Vitousek et al. 1997). It is a major component in both nucleic and amino acids. In plants it is a major component of chlorophyll and without it, photosynthesis could not take place. It is believed that before human intervention, N was a limiting growth agent to plants and in equilibrium throughout the globe (Vitousek et al. 1997). While the quantity of pre-human reactive N is unknown, it is estimated that anthropogenic N inputs from 1860 to 2000 increased from 15 Tg/yr to 165 Tg/yr, and this trend is expected to continue (Galloway 1998, Galloway et al. 2003). In addition, humans have effectively doubled the natural N cycle through the cultivation of leguminous crops, fertilizers, and energy production (Kinzig and Socolow 1994, Vitousek et al. 1997, Galloway 1998, Galloway et al. 2003, Galloway et al. 2008, Schlesinger 2009).

The largest inputs of anthropogenic N come in the form of fertilizers applied to agricultural ecosystems. Approximately 75%, or 120 Tg, of anthropogenic N is applied to agroecosystems per year, of which only 50% is estimated to be taken up by the intended plants (Galloway et al. 2003, Galloway 1998). The remaining applied N is lost to surface runoff, leaching and/or volatilization (Galloway 1998). Reactive N losses to surface or ground water undergo one of four possible fates: sediment storage, volatilization, translocation, or plant uptake (surface water only); however, in impaired water systems, significant N inputs can accelerate eutrophication events and/or affect pH, both of which can have strongly negative affects on aquatic ecosystems. To date, increased N concentrations are considered the greatest pollution problem in U.S. coastal waters, to the extent that one-third of these waters have been classified as "severely degraded" while another third is classified as "moderately degraded" (Bricker et al. 1999, Howarth et al. 2000, Rabalais 2002).

Similar to N, phosphorus [P] is also an essential nutrient for all biological organisms. Although relatively rare in the Earth's crust, P is critical in intercellular energy transfer and RNA and DNA synthesis (Fillippelli 2008). P is also an important component in photosynthesis and metabolism in plants, aiding young tissues in the formation and propagation of root growth, flowering, fruiting and seed formation (Smil 2000). While human perturbations to the global P cycle have been less studied than those for N and carbon [C], estimates suggest that P mining has increased from 1 Tg/yr in 1930 to ~16.5 Tg/yr in 1988, and P extraction rates are expected to reach 30 Tg/yr by 2050 (Fillippelli 2008, Smil 2000). Although P can be found in multiple products (i.e., herbicides and detergents), the primary use for extracted P is fertilizer for food production. Smil (2000) estimates that a global input of 14 – 15 Tg P, in the form of synthetic fertilizer, in addition to 10 – 14 Tg of naturally occurring P from soil weathering, atmospheric deposition and organic material, results in an estimated global plant uptake of 11 – 12 Tg P and an estimated global loss of 13 – 15 Tg P. P has no known stable gaseous form and is relatively immobile, making runoff and erosion the primary transportation loss pathways for P. Once transported to surface waters, P is subject to one of three possible fates: sedimentation, translocation or uptake by aquatic plants. Recent evidence suggests that P inputs as small as 0.01 mg P / L can accelerate eutrophication events (Smil 2000). In fact, in one study, 10 μg P / L input to a 10 hectare lake with an average depth of 5 m increased turbidity and, decreased clarity from 9 m to 3 m (Gibson 1997). Inputs above 50 µg P / L have been shown to result in anoxic conditions in the hypolimnion, resulting in strong negative environmental and economic impacts (Smil 2000).

Like terrestrial plants, aquatic plants also require nutrients, especially N and P. In most cases, plant response to N increases as P availability increases (Smil 2000). However, there is a fine line between a nutrient-stable ecosystem and a nutrient-saturated ecosystem. Eutrophication – the over-greening or abundance of plant growth in aquatic ecosystems which is triggered by natural or anthropogenic saturation of nutrients – seems to be a good indicator of that line. In aquatic ecosystems, high nutrient availability fuels high rates of primary production. However, rapid rates of decomposition can deplete available oxygen, leading to an oxygen depleted environment which cannot support most vertebrates. The most striking examples of the negative consequence of this phenomenon are the "dead zones" which characterize many eutrophide coastal waters (Dorfman 2004). Recent evidence suggests that P can be a primary driver of eutrophication events, stimulating N-fixing organisms that are ubiquitous in aquatic systems (Smil 2000). Decomposition of N-fixing organisms often creates an influx of available N for aquatic plant species; thus, it has been suggested that N is not as growth-limiting as P for aquatic plants (Schindler 2008).

Traditionally, the working mantra concerning pollution has been, "The solution to pollution is dilution." Dilution in this sense has been historically provided by the nearest available surface waters. Water quality was loosely protected in the United States dating back to the late 19th century by Congressional acts such as the Rivers and Harbors Act of

1886, the Federal Water Pollution Control Act of 1948 and the Water Quality Act of 1965; however, when the Cuyahoga River in Cleveland, Ohio caught on fire in 1969, the topic took center stage in both the hearts of American citizens and politicians in Washington DC. The following years would see the development of the Environmental Protection Agency [EPA] and the Clean Water Act [CWA] in an attempt to ameliorate the growing water pollution problem.

The Clean Water Act, enacted in 1972, aims to protect our vulnerable surface water by establishing water quality standards which support basic uses such as: 1) Drinking, culinary use and food processing; 2) Aquatic life, including but not limited to fish, waterfowl and furbearers; 3) Recreational and aesthetic value; 4) Agricultural needs, and 5) Industrial needs. In addition, the CWA defines “point source pollution” as a “discrete conveyance” such as a ditch or pipe discharging into a receiving water body. The CWA also defines “non-point source pollution” as a “diffuse source” (such as runoff from agroecosystems) which has the potential to move natural and anthropogenic pollutants from land to waters. Section 303(d) of the CWA mandates that the EPA and states work together to define total maximum daily loads [TMDL] – the daily allowable input of pollutants applicable to regulated water quality standards which ensures continued protection of a natural and balanced population of native shellfish, fish and wildlife – for any U.S. waterway listed as “impaired.” These TMDLs are then divided amongst point sources, as “waste load allocations,” and non-point sources, as “load allocations.” While current EPA regulations require point source polluters to closely monitor various waste load parameters, such as metals, biological oxygen demand, pH, total suspended sediments, and concentrations of N and P, neither the CWA nor the EPA have outlined regulatory legislation regarding non-point pollution sources. Inevitably, as non-point pollution sources increase due to population growth, the EPA will mandate stricter outflow regulations on point source pollutions. An emerging solution this growing problem is the development of watershed Voluntary Nutrient Reduction Plans [VNRP]. VNRPs are implemented by watershed-sharing communities to reduce nutrient inputs before the onset of state and federal mandated regulations, and are favored by communities that would rather self-manage water quality issues than be regulated by state and federal governments. Currently, VNRP development in rural communities may be the only method of reducing non-point pollution sources.

Nationally, wastewater treatment plants [WWTP] rank second in the top ten major pollution sources to surface water (Bricker 1999), and N and P rich inputs from WWTPs have been linked to eutrophication events (Kinzig and Socolow 1994, Vitousek et al. 1997, Carpenter et al. 1998, Peterson et al. 2001, Galloway et al. 2003, Galloway et al. 2008, Schlesinger 2009). Additionally, the EPA reports that WWTPs are the largest known cause of impaired estuaries, contributing 37% of reported impairment. The average age of WWTP in the United States is approximately 33 years; however, some existing facilities utilize systems nearly 200 years old (Dorfman 2004). In 2000, the EPA announced that without substantial increases in both investment and treatment efficiency in publicly owned treatment works (POTW, or WWTP) by the year 2025, U.S. waterways may return to sewage-related pollutant loadings similar to those of 1968, the highest in U.S. history. Arguably, nearly all treatment practices currently in place nation-wide will eventually need to be replaced. Furthermore, Dorfman (2004) suggests that the costs of repairing and upgrading facilities are minute relative to estimated environmental and economic costs, which range in the billions. In many cases, cities will have to utilize tax dollars for the construction of tertiary treatment facilities to further treat wastewater and ultimately meet current and future EPA mandates. There is an emerging concern, however, that smaller, less funded communities will be unable to afford such upgrades (Schroder et al. 2007). To date, while some treatment methods meet EPA discharge regulations, all continue to discharge detectable nutrient concentrations into receiving surface waters. One possible way to solve this problem is to begin viewing nutrient outputs in wastewater as wasted resources – resources that could alternatively be used to enhance degraded riparian zones, remediate soils, and restore wildlife habitat by utilizing natural ecological cycles already in place.

After the enactment of the CWA in 1972, the standard wastewater treatment method utilized by U.S. WWTPs has been comprised of preliminary, primary, and secondary treatment phases. During the preliminary treatment phase, influent is passed through a series of bar screens and grit channels to remove large particulate matter. The influent then enters a primary treatment phase, which consists of large circular holding tanks that promote the removal of all floating and sinking waste matter. These initial two treatment phases remove roughly 50% of the incoming organic matter. As the primary treatment cycle completes, the influent (termed "primary effluent") enters the secondary treatment phase. The secondary treatment phase employs a diverse community of microorganisms which promote the biological degradation and transformation of dissolved and colloidal organic compounds (Matas 2000). These microorganisms bind together suspended sediments, promoting flocculation, and creating activated sludge. Inside the activated sludge matrix, pollutants and nutrients are partially decomposed by nitrification/denitrification, biological mineralization, aeration, and/or, in the case of P, retained by bacteria (Schroder et al. 2007). A final filtration stage removes the activated sludge and effluent then undergoes a sterilization phase (i.e., chlorination or ultra violet radiation) and is discharged, in most cases, into the nearest body of water. N and P removal in this treatment method is approximately 63% and 65% respectively (Dorfman 2004) but roughly 37% N and 35% P remain in the effluent and are directly discharged into surface water.

Some proposed alternatives for tertiary wastewater treatment consist of membrane techniques, advanced oxidation processes, urine separation approaches, and others. Unfortunately, many of these proposed methods call for unreasonably high pretreatment requirements, unrealistic lowered throughputs, and/or impart an economic input equal to that presented by additional plant construction (Schroder et al. 2007). While these effective but costly methods may further reduce nutrient concentrations, one question remains: How will smaller, less funded communities bridge the ever-widening cost gap? Moreover, many of these suggested methods contain many of the same pitfalls associated with traditional wastewater management – less than 100% nutrient removal – providing only a temporary fix to a permanent problem. Ironically however, these techniques focus on removing and wasting nutrients, the same nutrients U.S. farmers collectively spend millions of dollars on annually. Further investment in research and development will prove useful in finding ways to fully utilize these sometimes scarce and overlooked resources.

Over the last 20 years, a number of new strategies for mitigating nutrient-rich wastewater have emerged. Much of this research has focused on management and use of biosolids – the solid waste obtained in the treatment process – as a fertilizer source, while only sparse research has been conducted on the effectiveness of vegetation filters used in tertiary effluent treatment. However, poplar stands have been found to retain 99% of N entering riparian areas, spurring the development of short-rotational hybrid poplars as vegetation buffers between agricultural fields and surface water systems (Haycock and Pinay 1993). Similar results were found in a 1998 study which recorded 95% N removal by tested riparian buffer zones examined in the Netherlands (Hefting and de Klein 1998). Furthermore, N uptake by fertilized poplar species may far exceed that by natural, non-fertilized poplars (Naiman and Decamps 1997).

There has also been increasing interest in the use of short rotational crops [SRC] as a method of tertiary wastewater treatment (Bond 1998, Moffat et al. 2001, Cavaleri et al. 2004, Lteif et al. 2008). This innovative tertiary effluent treatment offers cities a plausible and cost-effective treatment plan with an additional benefit of return funds resulting from harvested trees and potential carbon sequestration credits (Gasol et al. 2008). In addition, this type of system offers further nutrient removal by utilizing microbial activity present in soil ecosystems while creating a nutrient source for plant growth. The thought process behind this type of treatment is simple: Instead of discharging nutrients into a water body, this tertiary and final stage of treatment discharges nutrient-rich effluent directly onto a land area occupied by trees. This method offers the potential for nutrient uptake into plant biomass, microbial transformation and immobilization, and storage in the soil strata itself as a method of natural nutrient reduction, immobilization, and removal. Essentially, this method mimics a septic system, albeit on a much larger scale and the inclusion of specific vegetation which promotes rapid nutrient and water uptake.

Studies have also been performed to examine the nutrient uptake ability of hybrid poplars in response to various fertilization types. A study comparing poplar nutrient uptake in response to sewage sludge versus effluent fertilization concluded that nutrient uptake was more efficient given an effluent application rather than biosolid application (Moffat et al. 2001). Lteif et al. (2008) concluded that poplars are more efficient in retrieving nutrients from organic non-mineralized fertilizers compared to inorganic mineralized fertilizers – i.e., manure or effluent vs. synthetic fertilizer. Furthermore, a greenhouse study showed hybrid poplar nutrient uptake, represented by biomass accumulation, was greater in non-mineralized fertilizers as compared to mineralized sources (Cavaleri et al. 2004).

Much of the research on using WWTP by-products – effluent and biosolids – as a form of fertilization for SRC has been conducted outside of the U.S.; however, there is one example from North America in Woodburn, Oregon. Proclaimed as the "First in the Nation," the Woodburn Wastewater Treatment Plant began exploring a nutrient reuse method when they exceeded Oregon state and federal TMDLs (Ch2mHill 1998). Currently, Woodburn utilizes 140 acres of hybrid poplars (*Populus trichocarpa* X *Populus deltoides*) to efficiently treat approximately 1 million gallons of effluent per day during the growing season (Stultz 2009). The city plans to increase its plantation size to 338 acres by the year 2020 – large enough to treat all of the city's effluent during the growth season (Ch2mHill 1998). Unfortunately, little research has been conducted to estimate the total amount of nutrients captured and used by the system.

Given the current lack of understanding and data describing the effective use of Poplar plantations as a tertiary effluent treatment method, the primary objective of my study was to develop a Poplar plantation in Missoula, MT, and to use effluent as a water and fertilization source. Within the overall objective, I addressed two important questions: What effects does effluent application to poplar plantations have on soil? What effects does effluent application have on groundwater chemical properties? To address these questions, I established an experimental poplar plantation at the Missoula Wastewater Treatment Plant [MWTP] in Missoula, MT. To further address these questions, I monitored effluent nutrient concentrations, tree survivorship and growth, and soil and groundwater chemical properties throughout the first growing season following the establishment of the plantation in 2009.

Study Area

The Missoula Wastewater Treatment Plant [MWTP] (Figure 1) was initially built in 1976, upgraded for larger capacity in 1985, and upgraded in 2003/2004 to increase capacity and to install a tertiary Biological Nutrient Removal [BNR] facility (MDEQ 2006). The most recent BNR upgrade increased nutrient removal efficiency from approximately 50% to 75%. The MWTP currently serves approximately 63,000 people – including city residents, businesses and industries – and discharges treated effluent at a rate of approximately 8.5 mgd into the Clark Fork River via "Outfall 001" (MTDEQ 2006). Discharge characteristics for this outfall are included in Appendix A.

The Clark Fork River is currently on the CWA 303d Impaired Rivers List, most notably impaired in the following areas: aquatic life – including but not limited to cold water fishery habitat; primary contact recreation – including but not limited to swimming and kayaking; and culinary or drinking uses (MTDEQ 2008). In 1998, the Montana Department of Environmental Quality [MTDEQ] in conjunction with the Tri-State Implementation Council's Nutrient Target Subcommittee [TSIC] – a stakeholders group comprised of members from Montana, Idaho and Washington – developed a VNRP, which focused on identifying and reducing nutrient inputs to the Clark Fork River basin (TSIC, 1998). This VNRP was submitted to and accepted by the EPA as a functional equivalent of a TMDL in September 1998 (Appendix B). Under this VNRP, the MWTP is limited to discharge effluent concentrations of approximately 1 mg P / L and 10 mg N / L into the river from Outfall 001.

In 2006, the MTDEQ issued the first Discharge to Land Permit in Montana. “Outfall 002,” as it has been named in the permit, is located at approximately 46°52’53” N latitude, 114°02’07” W longitude and is the target area of our study. This study area consists of an approximate two-acre plot located on the southern border of the City of Missoula Wastewater Treatment Plant (Figure 1). Soils at the site are described as Orthents (Entisols), which are very deep, well drained to excessively drained soils formed in a variety of disturbed and reworked soils (USDA 1994), but the substrates could be more accurately described as a mix of homogeneous overburden of alluvial origin (resulting from previous disturbances during prior land use activities). Soil texture at the site is classified as loamy sand with 1% soil organic matter and a pH of 7.4 ± 0.5 in the surface 0-10 cm. The site is situated on a Clark Fork River terrace, approximately 4.5 m above peak flow height, with slopes 0 to 4 percent, and elevation at the site is approximately 1000 m ASL. Annual precipitation ranges from 28 to 36 cm, the frost-free season is 105 to 120 days (USDA 1994), and mean annual temperature is 6.8 °C.

Prior to treatment plant construction in 1976, the study area served as a landfill. This site also served as a construction staging area during the two previously mentioned plant upgrades in 1985 and 2003/2004 (MTDEQ 2006). Approximately 0.4 acres – originally proposed as a control plot – east of study area were restored to simulate native prairie in 2004 by Rocking M Design, PC. This restoration included the introduction of 1000 pounds of mulch and a native grass seed mix composed of five native species (Table 1). The remaining 1.6 acres – allotted to effluent application – were left idle and thus invaded by multiple native and nonnative weeds (Table 2). Prior to planting, this weed infested area was sprayed with Roundup weed killer during fall 2008. Following herbicide application, approximately 500 m^3 of EKO compost was spread evenly throughout the western 1.6 acres. The site was left in this fallow condition for the 2008/2009 winter period.

Figure 1. An overview of MWTP. The western non-restored area, bordered in red, is approximately 1.6 acres and represents the area designated as Outfall 2. The eastern restored area, bordered in yellow, is approximately 0.4 acres and represents the originally proposed control plot. Site differences are clearly visible.

Table 1. Composition of native plant species introduced by Rocking M Design, PC in 2004. Applicable area is bordered in yellow in Figure 1.

Species	
Scientific Name	**Common Name**
Agrophyron smithil	Western Wheatgrass
Agrophyron spicatum	Bluebunch Wheatgrass
Festuca ovina	Sheep Fescue
Poa sandbergii	Sandberg Bluegrass
Puccinella distans	Alkaligrass

Table 2. Invasive, nonnative and native plants species found within the area designated as Outfall 002. Applicable area is bordered in red in Figure 1.

Species	
Scientific Name	**Common Name**
Patulaca oleracae	Common Purslane
Panicum capillare	Witchgrass
Eragrostic minor	Little Lovegrass
Kochia scoparia	Burning Bush
Amaranthus albus	Tumble Weed
Convolvulus arvenisis	Field Bindweed
Amaranthus retroflexus	Redroot Amaranth
Erodia cicutarium	Redstem filaree
Hyoseyamus niger	Henbane
Sisymbrium altissimum	Tumble Mustard
Chenopodium album	Lamb's Quarter
Sataria lutescens	Barngrass
Taraxacum laevigatum	Rock Dandelion
Euphorbia esula	Leafy Spurge
Chenopodium botrys	Jerusalem Oak
Solanum dulcamara	Deadly Nightshade
Euphorbia serphyllifolia	Thyme-leafed Spurge
Tanacetum valgare	Tansy
Cirsium arvenge	Canadian Thistle
Potentilla recta	Sulphur Cinquefoil
Gaillardia aristata	Common Gaillardia
Verbena bracteata	Bigbract Verbena
Oenothera strigosa	Hairy Evening Primrose
Centaurea stoebe	Spotted Knapweed

Of special concern in this project is the Missoula Aquifer, which has been defined by MTDEQ as the receiving water body for effluent discharged from Outfall 002. The EPA has defined the Missoula Aquifer as a sole or principal source aquifer – "an aquifer that supplies at least 50 percent of the drinking water consumed in the area overlying the aquifer … and has no alternative drinking water sources" (MTDEQ 2006). A study performed in 1988 suggested that ground water in the vicinity of Outfall 002 primarily flows west to south where it reenters the Clark Fork River (Woessner 1998) (Figure 2).

Figure 2. Groundwater flow patterns as suggested by Woessner, 2008 are depicted above. The study area, Outfall 002, has been outlined in red. Numbers indicate elevation.

Study Design

In April 2009, forty two rectangular plots, each measuring approximately 90 m^2, were established in the effluent irrigation treatment area. In addition to the effluent irrigation treatment plots, I also created four plots of the same dimensions to serve as groundwater irrigation treatment plots which would function as experimental controls. These two plot areas were separated from one another by a "no irrigation" buffer zone (Figure 3). An eight foot high fence was also installed around the perimeter of the study area to minimize herbivory within the treatment plots.

Tree Planting and Treatment Implementation – Two cold-tolerant hybrid poplar species (*Populus deltoides* X *P. trichocarpa* and *P. deltoides* X *P. nigra)* and one native poplar (*P. balsamifera* ssp. *Trichocarpa*) were selected for this study. Hybrid Poplars were ordered from Segal Ranch, Idaho and received as 46-cm un-rooted whips. Native Poplar un-rooted whips, approximately 46 cm, were collected from Kelly Island, Montana in February 2009 and stored at 4°C. In addition to Poplar species, I also planted 20 *Pseudotsuga menziesii*, 20 *Pinus ponderosa* and 10 *Abies grandis* outside the study for observational analysis only (data not included in this report).

Trees were planted between 5 and 10 May 2009. Planting consisted of pounding a 1.25 m steel stake approximately 31 cm into the soil using a fence post driver, boring the hole by rotating the stake, and placing one un-rooted whip into each hole. Loose soil was then placed back into the hole around the tree and approximately 1 L of ground water was applied immediately after planting.

Figure 3. A planted un-rooted Poplar whip before irrigation installation and mulching.

Poplar species were equally and randomly assigned to each effluent treatment plot. Six trees of each species were planted 4.25 m X 4.25 m apart in each of the 42 effluent plots, resulting in a total of six trees per plot (Figure 6). A similar selection and planting process was used to plant trees in the groundwater treatment plots. However, since there were only four groundwater plots, I planted *P balsamifera* ssp. *trichocarpa* in two plots (12 *P balsamifera* individuals in total) while *P deltoides* X *P trichocarpa* and *P deltoides* X *P nigra* were each planted in one of the remaining two groundwater plots (six individuals of each species in total). In addition, six trees of each species were planted in the no- water buffer plot. Extra trees of each species were planted in pots to be used as replacements for trees that did not break bud. Finally, following planting, approximately 5 ml of Plant Skydd, a bovine/porcupine blood mixture, was applied to the stems and emergent leaves of all trees to further prevent herbivory within the treatment area.

Identical irrigation systems were installed in the effluent irrigation and groundwater irrigation study areas on 7 May 2009 and 4 June 2009, respectively. Each irrigation system consisted of a 9-mm diameter high density, polyethylene irrigation line installed along each row (South to North) of trees. Individual 10.5 gallon/hour micro spray irrigation heads were installed within 30 to 60 cm of each tree. Spray heads were inverted to focus effluent irrigation directly onto the base of each tree.

Figure 4. A photo of an installed irrigation line. The wooden board on the right is covering a soil monitoring hole (to be discussed later in this document). A *Pinus Ponderosa* occupies the foreground.

The effluent irrigation system became functional on May 7, 2009 and was set to irrigate each day from 9:00 - 9:36 am at a rate of approximately 2041 gallons per day applied evenly to all trees within the effluent treatment plot. Watering times and durations for the groundwater irrigation system were identical to those in the effluent irrigation system. Under this irrigation regiment, trees received approximately 6.3 gallon/day of effluent or groundwater, dependant upon treatment plot.

Irrigation timing and duration followed restrictions set by the MTDEQ Land Application permit, which allowed a hydraulic loading rate of 280,000 gallons of treated effluent irrigation in 2009 (Appendix 3). The hydraulic loading rate was based upon Idaho Department of Environmental Quality's *Guidance for Land Application of Municipal and Industrial Wastewater* (2004).

Following irrigation installation and tree planting, approximately 0.12 m^3 (roughly a 115 cm diameter 7.62 cm thick layer) of freshly processed wood mulch was placed around each tree in an attempt to decrease moisture loss from evaporation. Mulch was primarily composed of *Pinus ponderosa*; however, it also contained various amounts of Cedar, Fir, Maple and Spruce tree species. Similar amounts of fresh mulch were also placed around each soil monitoring hole (See Soil Sampling and Analysis Section) to simulate potential changes in soil properties resulting from the addition of mulch.

Given the low application rates allowed in year 1 under the application permit conditions, the allotted "irrigation water requirement" [IWR] was reached by 25 July 2009. As a result, effluent irrigation ceased on that date, and trees within the effluent irrigation study area were then irrigated with groundwater until September 2, 2009, when the irrigation system was turned off to promote leaf senescence and tree dormancy.

Figure 5. A replicate of Figure 4 albeit the completed mulch application.

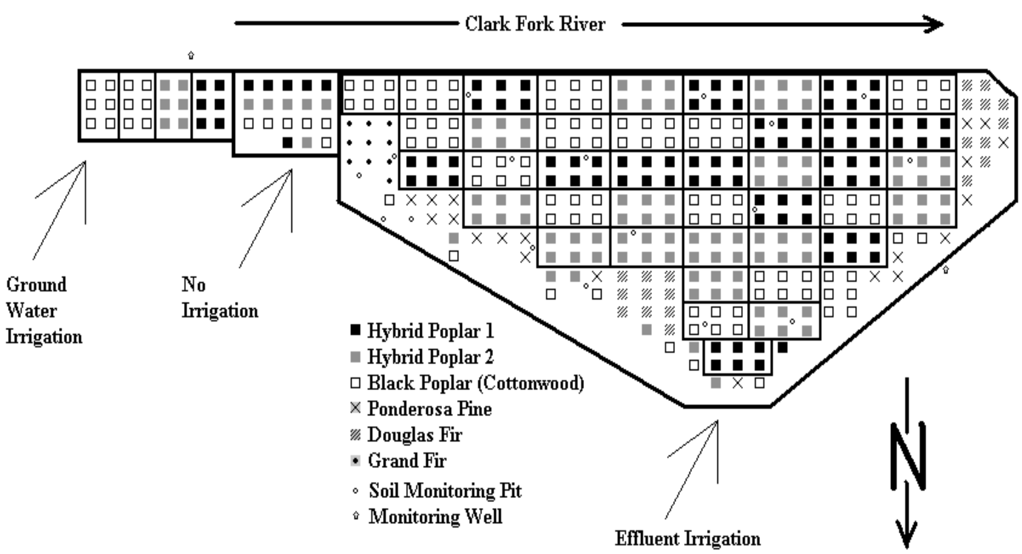

Figure 6. Plot layout: Plots are 90 m^2 and each contains six trees of the same species, spaced 4.25 m X 4.25 m apart. 10.5-gph micro-spray irrigation heads were placed approximately 31 to 61 cm from each tree base. Soil monitoring pits and ground water monitoring wells are noted in the figure, as are individual treatment areas. Drawing is not to scale.

Materials and Methods

The main objective in this study was to assess whether or not I could successfully develop an effective Poplar plantation irrigated with treated effluent in Missoula, MT. To that end, I monitored effluent nutrient concentrations, tree survivorship and growth, and soil and groundwater chemical effects throughout the study period (May – September 2009).

Treated Effluent Sampling and Analysis – Effluent chemical properties were measured from April 2009 to September 2009 – before, during and after treatment. Effluent samples were obtained via a 24-hour flow paced sampling system, which consisted of an automated in situ grab of approximately 100 to 150 mL occurring on average of 3 times per hour. The in situ grab sampling events resulted in an ~7200 mL composite sample constructed over time. Samples were obtained at the very end of the treatment cycle, after UV sterilization had occurred. In the event that samples were not immediately analyzed, they were refrigerated at 4°C. To prevent any possible microbial transformations, samples that could not be analyzed within 24 hours were also preserved with sulfuric acid to a pH less than 2 (approximately 2 mL conc. H_2SO_4 per liter sample). Samples for P analyses were immediately filtered through a 0.45-µm membrane, refrigerated and analyzed within 48 hours. Effluent chemical analysis was performed in adherence with the EPA approved, 21st Edition of *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al. 2006). Effluent analysis was performed by the MWTP at their on-site analytical laboratory.

Effluent Total P [TP] and Total Kjeldahl Nitrogen [TKN] concentrations were analyzed three times per week – generally occurring on Monday, Wednesday and Friday.

TP sample preparation utilized an ascorbic acid reduction coupled with a manual digestion process which was then colorometrically analyzed via an automated flow injection system – method 4500-P H (Greenberg et al. 2006). TKN sample preparation consisted of a block digestion followed by automated flow injection colorometric analysis – method 4500-Norg D (Greenberg et al. 2006).

Total Suspended Sediment [TSS], Chemical Oxygen Demand [COD], and Biological Oxygen Demand [BOD] were analyzed two times weekly. During the treatment phase (7 May 2009 through 25 July 2009) these samples were obtained twice per week from randomly selected irrigation heads and immediately analyzed. TSS monitoring methods consisted of filtering the effluent sample through a 1.2-µm filter, drying the filter at 103 – 105 °C, and then calculating the associated filter weight difference – method 2540-Solids D (Greenberg et al. 2006). COD samples were prepared using a dichromate closed reflux method and analyzed colorometrically – method 5220-COD D (Greenberg et al. 2006). BOD samples were analyzed using a 5-day BOD test – method 5210-BOD B (Greenberg et al. 2006).

Soluble Reactive P [SRP], Ammonium [NH_4^+], Ammonia [NH_3], Nitrate [NO_3^-], and Nitrite [NO_2^-] in effluent were analyzed once per week. SRP samples were prepared with an ascorbic acid reduction followed by automated flow injection colorometric analysis – method 4500-P G (Greenberg et al. 2006). NH_4^+ and NH_3 samples were prepared with a phenate method and then colorometrically analyzed using an automated flow injection system – method 4500-NH_3 H (Greenberg et al. 2006). NO_3^- and NO_2^- samples underwent cadmium reduction before automated flow injection colorometric analysis (Greenberg et al. 2006).

Effluent pH and Electric Conductivity [EC] were measured during each sampling event. pH analysis was performed via the electrometric method – method 4500-H+ B (Greenberg et al. 2006). EC values were measured with a conductivity cell – method 2510-C B (Greenberg et al. 2006).

Tree Sampling and Analysis – Tree sampling occurred on 2 June, 13 July, and 2 September of 2009. During each sampling event, all trees were measured for height and survivorship. Height data were collected using a retractable 3.5-meter measuring tape, a meter stick, and/or an incremented 300-cm pole depending on the overall height of the tree. Survivorship data were collected by noting whether trees showed signs of life – i.e., leaves and/or sprouting branches. Trees displaying limited or no signs of life were replaced with another individual of the same species of tree from the original group of cuttings. Replaced trees were studied for continued survivorship; however, for analysis purposes, the tree was considered dead in survivorship calculations.

Soil Sampling and Analysis – Eighteen soil monitoring holes were machine augered in random locations within the study site in April 2009 prior to tree planting. Monitoring hole diameters ranged from 0.5 to 1 m and depths ranged from 1 to 2 m. Monitoring holes were encircled by mulch as noted above and covered with 1.5 m^2 plywood boards for safety and to prevent evaporation. Soil samples throughout the duration of this project were obtained from two depths within each hole: 0 to 30.5 cm and 30.5 to 61 cm, respectively. A composite sample for each of the two depths was then created by combining samples from at least 15 of the 18 monitoring holes. These soil monitoring methods, established by Mahler and Tindall (1997), were chosen and required under the permit established by MTDEQ (2006). Soil samples were sieved through a 2-

mm mesh and sealed in air-tight plastic ziplock bags immediately after collection. Samples were then shipped to Energy Labs in Billings, MT for analysis.

Soil sampling occurred on April 8th, June 3rd, and September 8th of 2009 (before, during, and after treatment). Each soil composite was analyzed for percent moisture, pH, TP, TN as NO_3^- and NO_2^-, TN as NH_4^+ and NH_3^+, TKN, Sodium Adsorption Ratio [SAR] and EC. Soil analyses were performed in coherence with the EPA approved methods set forth by the USDA Handbook 60 (USSLA 1954), EPA Test Methods for Evaluating Solid Waste (EPA 1995), and Soil Science Society of America's Methods of Soil Analysis (Page 1982).

Percent moisture values were obtained using the Gravimetric soil moisture method. In this method, field moist soil samples were weighed, placed in a drying oven at 100°C for 24 hours, and reweighed to determine moisture loss – method D2974 (USSLS 1954). Gravimetric soil moisture was calculated as the ratio of water (moist soil – dry soil) to dry soil.

EC and pH samples were prepared using a saturated paste method (Page 1982). The resultant extract was then measured for EC and pH with an EC reader and pH meter respectively – methods ASAM10-3 and ASAM10-3.2 (Page 1982).

SAR, a ratio of sodium [Na] to the combination calcium [Ca] and magnesium [Mg], was also prepared using a saturated paste method (Page 1982). Filtrate samples were then analyzed using inductively coupled plasma mass spectrometry – method SW6010B (EPA 1995). The following equation was then calculated:

$$\text{SAR} = \frac{[Na^{+}]}{\sqrt{\frac{1}{2([Ca^{2+}]+[Mg^{2+}])}}}$$

where concentrations are in mmol/L

Soil inorganic nitrogen [IN] samples were analyzed using a potassium chloride [KCl] extraction – method ASA33 7&8 (Page 1982) – although TKN samples were processed using a Kjeldahl digestion apparatus – method ASA31-3 (Page 1982). Resultant extracts were then analyzed using an automated colorometric analyzer.

P samples were processed using block digestion – method 3050B (EPA 1995). Digest filtrates were examined using an automated colorometric analyzer.

<u>Groundwater Sampling and Analysis</u> – Two 50-foot monitoring wells were established in April 2009, before tree planting (Figure 7). As mentioned previously, groundwater in the vicinity of our study area moves west to south (Woessner 1988); thus, one well was located on the upper end of this gradient (i.e., before reaching the treatment plot) and the second was located down-gradient (i.e., after passing below the treatment plot) in an attempt to capture the potential effects of the effluent application on groundwater chemical properties.

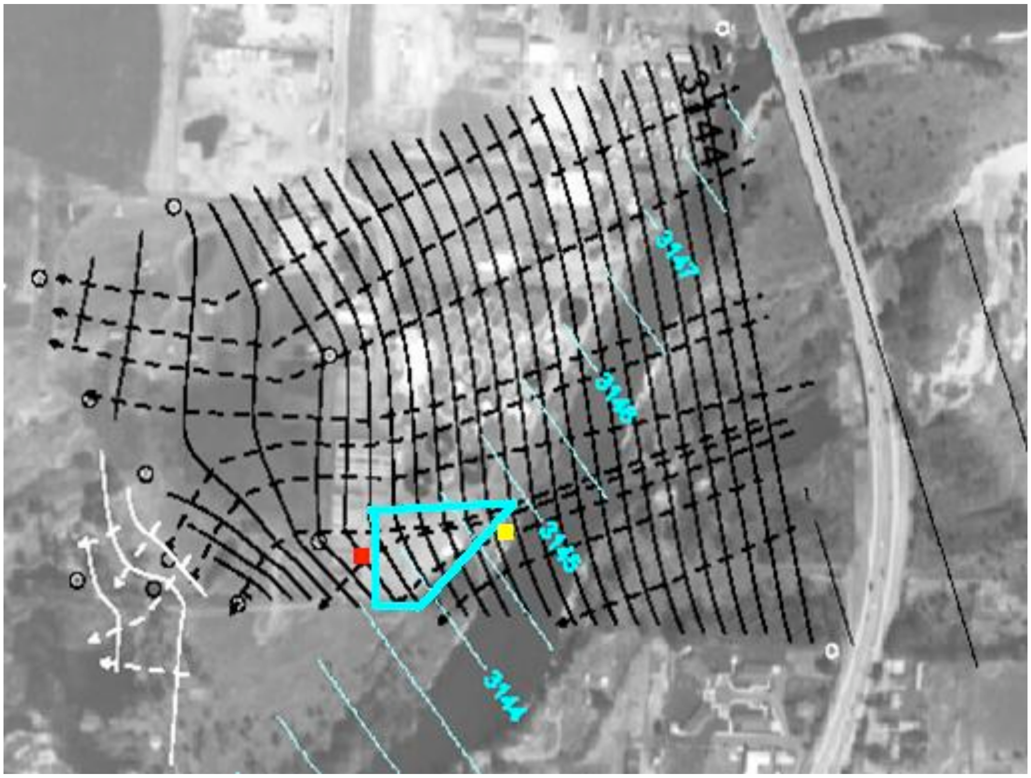

Figure 7. Placement of groundwater monitoring wells is depicted above. The study area has been outlined in blue. The up-gradient monitoring well is represented by the yellow square and the down-gradient monitoring well is represented by the red square.

Groundwater chemical attributes were measured in samples collected during eight sampling events from April 2009 to September 2009 (before, during and after treatment). All parameters – pH, EC, NH_4^+, NH_3, NO_3^-, NO_2^-, TKN, TP, and SRP – were monitored once per month, except in May and July when samples were collected twice per month. 1000-mL in situ samples were obtained using dedicated pump and sampling tubing. Each well was pumped for approximately 5 minutes or until the EC and temperatures of two consecutive samples were within 5% of one another. The sample bottle was rinsed with well water prior to sampling and then filled. Groundwater chemical analysis was also performed in adherence with the EPA approved, 21st Edition of *Standard Methods for the Examination of Water and Wastewater* (Greenberg et al. 2006). Sample processing and analyses were identical to those used in the Treated Effluent Sampling and Analysis section of this document. Groundwater analysis was conducted by the MWTP at their on-site laboratory.

Data Analysis

I explored collected data values to identify trends or patterns in soil and groundwater chemical properties resulting from effluent irrigation over the course of one growing season. In most cases these data are presented as time series graphs or data tables. Where variability among data values could be computed, I chose a Standard Error calculation represented by the standard deviation divided by the square root of the number of samples: $\sigma/\sqrt{n}$. This error calculation seemed appropriate as sample quantity varied per parameter throughout the project, and this method calculates variance among samples rather than variance from the mean. In all cases, Total organic N [TON] was calculated by subtracting NH_4^+ from TKN; Total inorganic N [TIN] was calculated as the sum of NH_4^+, NH_3, NO_3^- and NO_2^-; NH_4^+ represents of the sum NH_4^+ and NH_3; NO_3^- is representative of the sum NO_3^- and NO_2^-. Finally, TN and TP represent the sum of all N species and all P species, respectively.

Treated Effluent Data – Data values assessed more than once a week were averaged to obtain weekly means, and weekly means were used to calculate monthly averages. N and P data values were plotted on individual graphs to depict changes in chemistry throughout the period of this study. Values are reported in Table 3 and Figures 8 & 9 of this document.

Tree Data – Height values for each measurement event were averaged per species per treatment type to yield mean incremental growth. Survivorship rates for each species, regardless of treatment, were calculated at the end of the first growth season by dividing the sum of surviving trees by the sum of planted trees. Values are reported in Table 4 and Figure 10 of this document.

Results

Treated Effluent Chemical Properties – Average monthly effluent chemical properties from April 2009 through September 2009 are reported in Table 3. COD, BOD and TSS appear to have decreased over time, possibly due to increased effluent temperature after April.

Table 3. Effluent chemical properties through time. Data values represent means ± 1 SE. Values provided by MWTP.

Date	pH	Temp (°C)	COD	BOD	TSS
Apr-09	7.30 ± .07	12.86 ± .54	3.13 ± .15	5.56 ± .33	6.86 ± .29
May-09	7.18 ± .03	14.80 ± .31	2.22 ± .14	5.41 ± .34	4.45 ± .27
Jun-09	7.10 ± .02	15.40 ± .25	1.57 ± .05	5.06 ± .41	3.82 ± .37
Jul-09	7.32 ± .05	18.36 ± .51	1.67 ± .11	3.70 ± .47	2.66 ± .02
Aug-09	7.43 ± .02	19.91 ± .07	1.19 ± .08	4.02 ± .32	2.68 ± .26
Sep-09	7.31 ± .02	19.74 ± .28	1.37 ± .13	4.08 ± .58	3.18 ± .30

Average monthly effluent N concentrations are depicted in Figure 8. Total organic N [TON] (calculated by subtracting NH_4^+ from TKN) and NH_4^+ concentrations remained relatively constant over the course of the growing season, averaging 1.45 ± 0.13 mg/L and 0.20 ± 0.03 mg/L, respectively. NO_3^- concentrations also showed relatively little variability over the course of the growing season; concentrations ranged from 7.33 ± 0.36 mg/L in April to 4.38 ± 0.27 mg/L in June and then increased to 7.65 ± 0.69 mg/L in September.

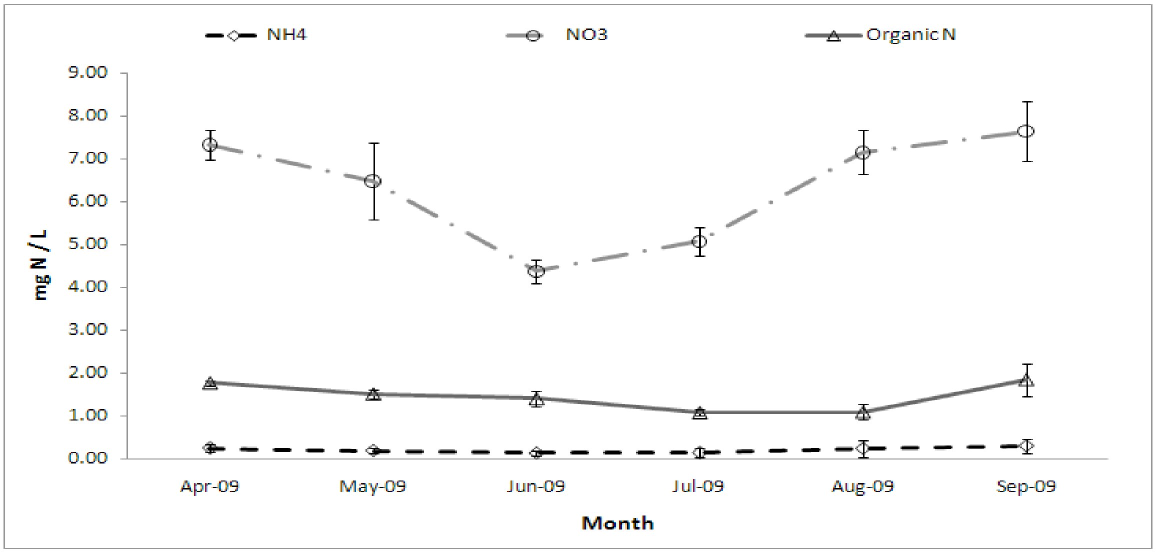

Figure 8. Effluent N averages through time. Data values represent means ± 1SE. NH4 represents the sum of NH_4^+ and NH_3. Values provided by MWTP.

Average monthly effluent P concentrations are depicted in Figure 9. Soluble P represents the concentration of orthophosphate [PO_4^{3-}], the form of P that is considered "plant available," while total P represents all forms of P – dissolved and particulate – including PO_4^{3-}. Similar to N concentrations, soluble P concentrations remained fairly constant over the growing season, averaging 0.31 ± 0.04 mg/L, albeit a small increase of 0.17 mg/L above the mean in July. Total P concentrations varied very little over the course of the growing season; concentrations ranged from 1.09 ± 0.13 mg/L to 0.26 ± 0.27 mg/L and then increased to 0.92 ± .26 mg/L in September.

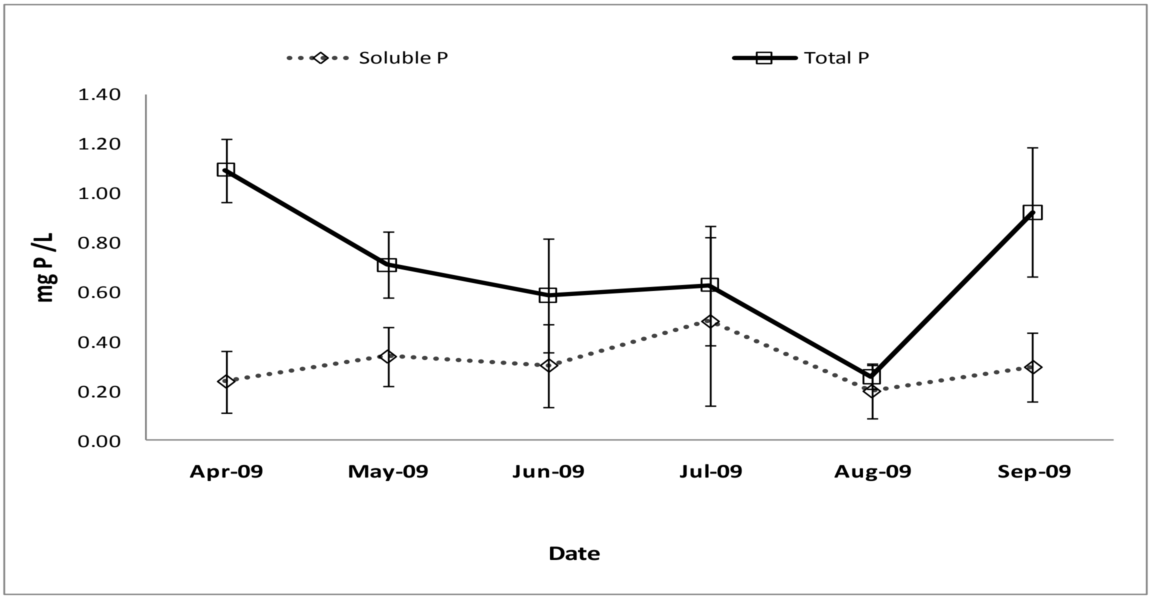

Figure 9. Effluent P averages through time. Data values represent means ± 1SE. Values provided by MWTP.

Tree Properties – Tree survival rates at the end of the first growing season were high (Table 4). Both *Populus* b*alsamifera* ssp. *trichocarpa* and *P deltoides* X *P nigra* had survival rates of 98%. *P trichocarpa* X *P deltoides* also had a high survivorship (94%).

Table 4. Tree survivorship resulting from effluent watering.

Species	Survival Rate (%)
P balsamifera ssp.trichocarpa	98
P trichocarpa X *P deltoides*	94
P deltoides X *P nigra*	98

Poplar species irrigated with effluent grew nearly twice as tall as Poplars irrigated with water alone, regardless of species. All three Poplar species responded positively to effluent (Figure 10). The data, at this point, do not suggest differences in growth response among Poplar species.

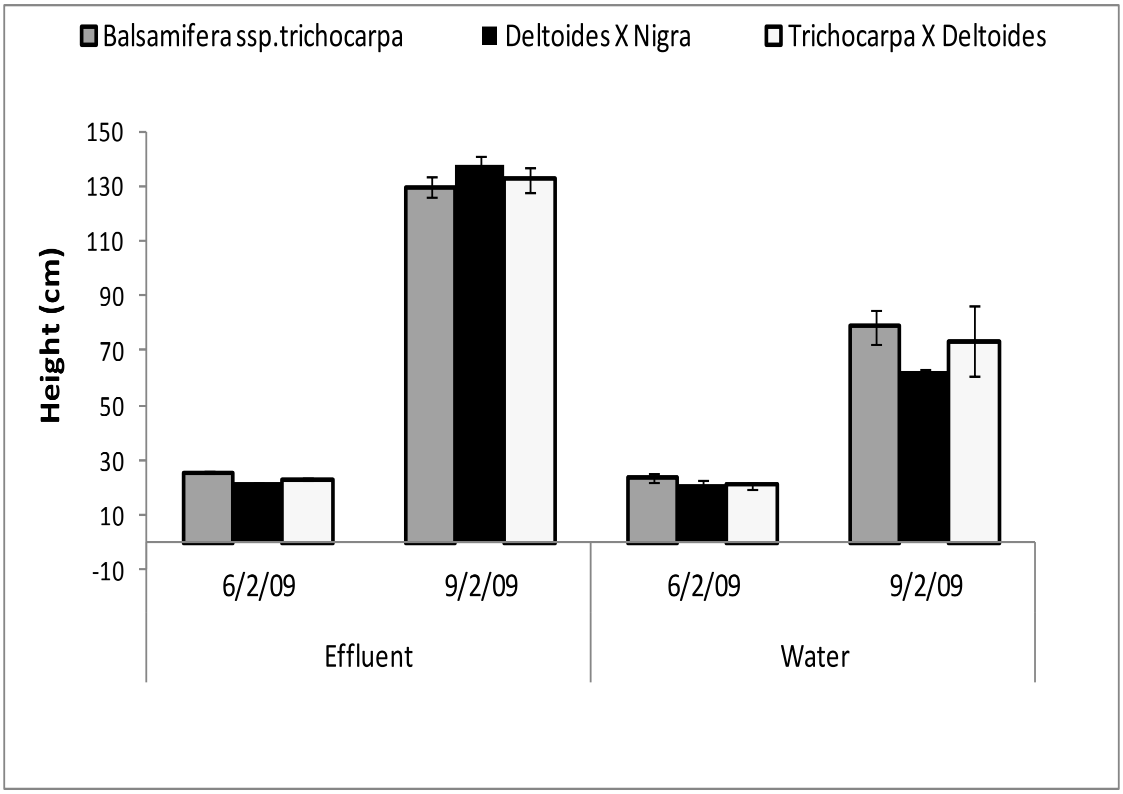

Figure10. Average tree heights per treatment type. Values represent means ± 1SE. Gray bars represent *Populus balsamifera* ssp.*trichocarpa* (Black Cottonwood), black bars represent *P deltoides X P nigra* (a hybrid) and white bars represent *P trichocarpa X P deltoides* (a hybrid).

<u>Soil Chemical Properties</u> – Quarterly soil chemical properties are reported in Table 5. An increase in Inorganic N as NO_3^- and NO_2^- was observed immediately following initial effluent irrigation. EC also increased from April to June, indicative of soluble salts present in the effluent. These levels decrease in September possibly resulting from the switch of effluent to groundwater irrigation in the effluent irrigation study area.

Table 5. Soil chemical properties through time. Values provided by Energy Labs in Billings, MT.

Date	8-Apr-09		3-Jun-09		8-Sep-09	
Sample Depth (cm)	0-30.5	30.5-61	0-30.5	30.5-61	0-30.5	30.5-61
pH	7.47	7.4	7.6	7.8	7.5	7.6
Total Organic N (mg N/kg)	950	950	721	780	837	613
Inorganic N (NO3- & NO2-) (mg N/kg)	10	8	77	24	66	23
Inorganic N (NH4+) (mg N/kg)	2	2	7	4	3	3
Sodium Adsorption Ratio [SAR] (meg/L)	1.62	1.47	2.91	1.82	2.08	1.76
Electrical Conductivity [EC] (mmhos/cm)	3.11	3.04	5.56	4.01	4.77	3.73
Soil Moisture (%)	12.9	14.1	8.7	14.3	11.5	13.9

Soil TN (Figure 11) values decreased over the duration of our study. It is important to note an initial increase in TIN concentrations in both soil depths possibly reflecting effluent irrigation (Table 5). However, these increases are overshadowed by a relatively large decrease in soil TON.

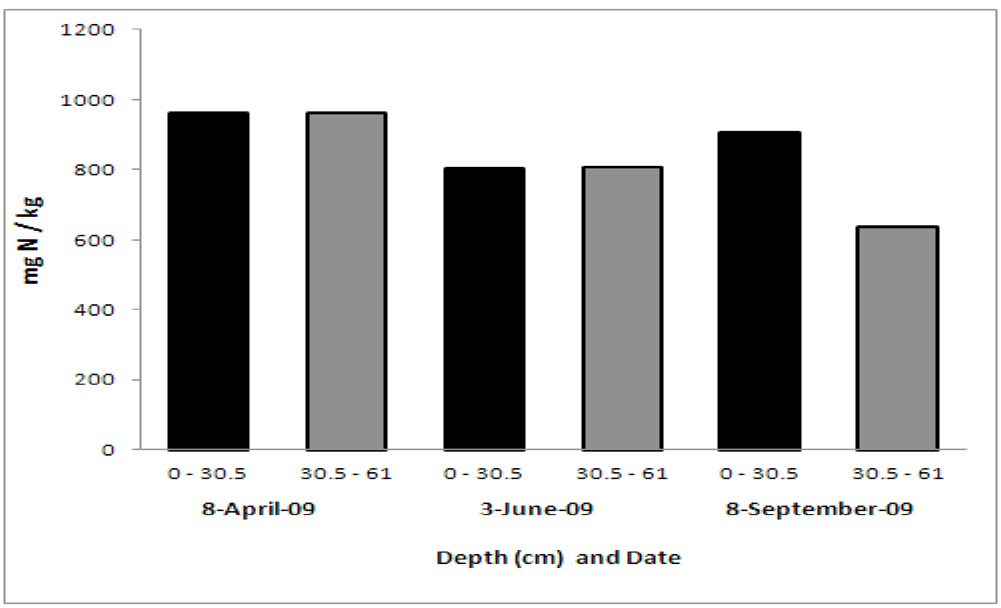

Figure 11. Soil TN values through time. Black bars represent soil composite samples taken from 0-30.5 cm depth. Gray bars represent soil composite samples taken from 30.5-61 cm depth. Values provided by Energy Labs in Billings, MT.

Soil TP concentrations through time are reported in Figure 12. TP concentrations in the 0-30.5-cm depth interval decreased throughout the entire growing season, from 1400 mg/kg in April to 1190 mg/kg in September. TP concentration in the 30.5-61-cm soil depth interval increased initially from 1440 mg/kg in April to 1740 mg/kg in June, followed by a decrease to 1490 mg/kg in September. Overall, TP concentrations in the 0-30.5-cm depth interval were lower at the end of the first effluent irrigation season than at the beginning, whereas initial and final TP concentrations in the 30.5-61-cm depth interval were nearly equal; 1440 vs. 1490 mg/kg in dry soil.

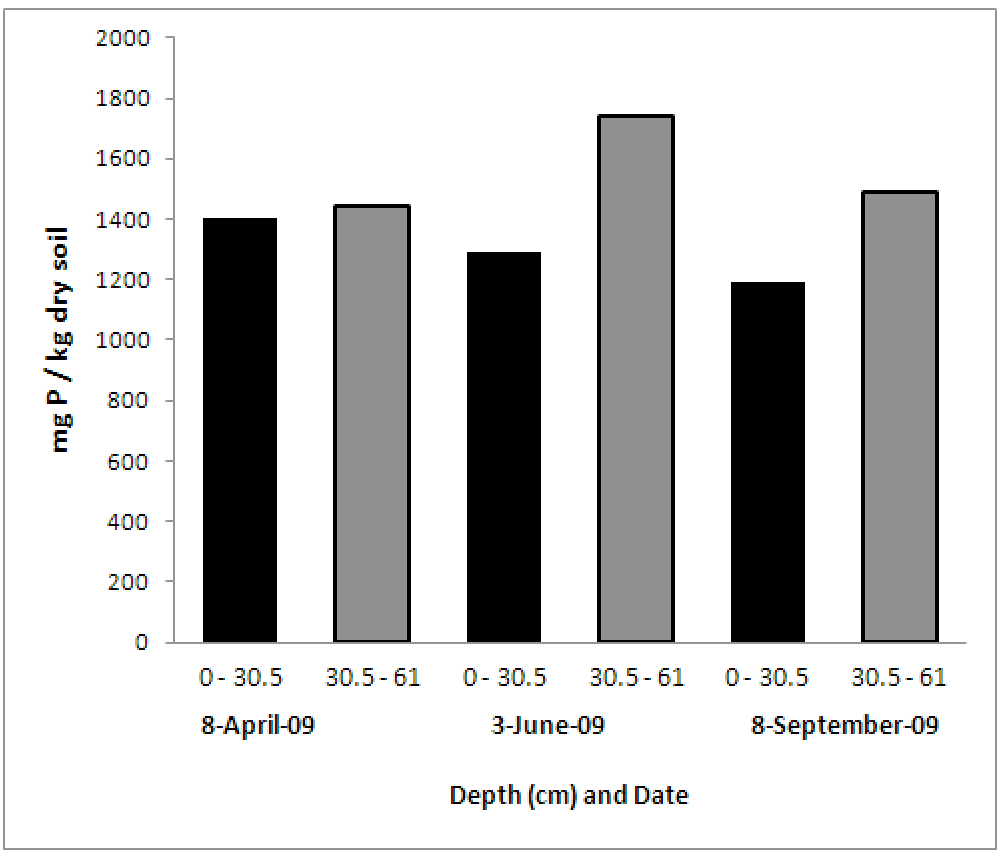

Figure 12. Soil TP through time. Black bars represent soil composite samples taken from 0-30.5 cm depth. Gray bars represent soil composite samples taken from 30.5-61 cm depth. Values provided by Energy Labs, Billings, MT.

Groundwater Chemical Properties – Groundwater chemical properties are provided in Table 6. Upper-gradient monitoring well values represent concentrations in the absence (or prior to) treatment application, whereas down-gradient monitoring well values represent concentrations that reflect treatment effects. With the exception of a slight increase in pH (i.e., 0.07), all parameters decreased over time, possibly indicating no affect to groundwater resultant from effluent irrigation.

Table 6. Groundwater chemical properties through time. Reported values represent the difference between monitoring wells. Positive values indicate an increase in groundwater chemical properties resultant from effluent treatment whereas negative values indicate decrease in groundwater chemical properties. Values provided by MWTP.

	Parameter						
Date	pH	Electrical Conductivity (mmhos/cm)	Total Inorganic Nitrogen (Nitrate + Nitrite + Ammonia) (mg N /L)	Total Kjeldahl Nitrogen (Organic + Ammonia) (mg N /L)	Total Nitrogen (Organic + Inorganic) (mg N /L)	Total Phosphorus (mg P /L)	Soluble Reactive Phosphorus (mg P /L)
30-Apr-09	0.04	61.00	0.31	0.26	0.57	-0.05	-0.03
22-May-09	N/A	N/A	-1.65	0.00	-1.65	-0.10	-0.10
28-May-09	0.25	41.00	0.01	0.00	0.01	-0.23	-0.06
24-Jun-09	0.03	-12.00	-0.07	0.00	-0.07	-0.06	0.33
08-Jul-09	0.20	-24.00	-0.12	0.00	-0.12	-0.06	-0.07
24-Jul-09	0.04	0.00	-0.04	0.00	-0.04	-0.04	-0.03
07-Aug-09	-0.42	27.00	-0.04	0.00	-0.04	0.00	-0.01
16-Sep-09	0.11	17.00	-0.02	N/A	N/A	N/A	N/A

Groundwater TN concentrations (Figure 13) represent the sum of all forms of N, inorganic and organic. An initial increase in up-gradient groundwater TN concentration was observed at the beginning of the treatment application, from 1.07 mg/L on April 30 to 2.93 mg/L on May 22. This concentration decreased to 1.02 by May 28, then reached and remained fairly constant at an average of 0.67 ± 0.33 mg/L by June 24 through August 7. Down-gradient TN concentrations decreased initially from 1.64 mg/L on April 30 to 1.03 mg/L on May 28, then decreased to and remained fairly constant at an average of 0.60 ± 0.30 mg/L by June 24 through August 7. Groundwater TN concentrations appeared to be unaffected from effluent irrigation.

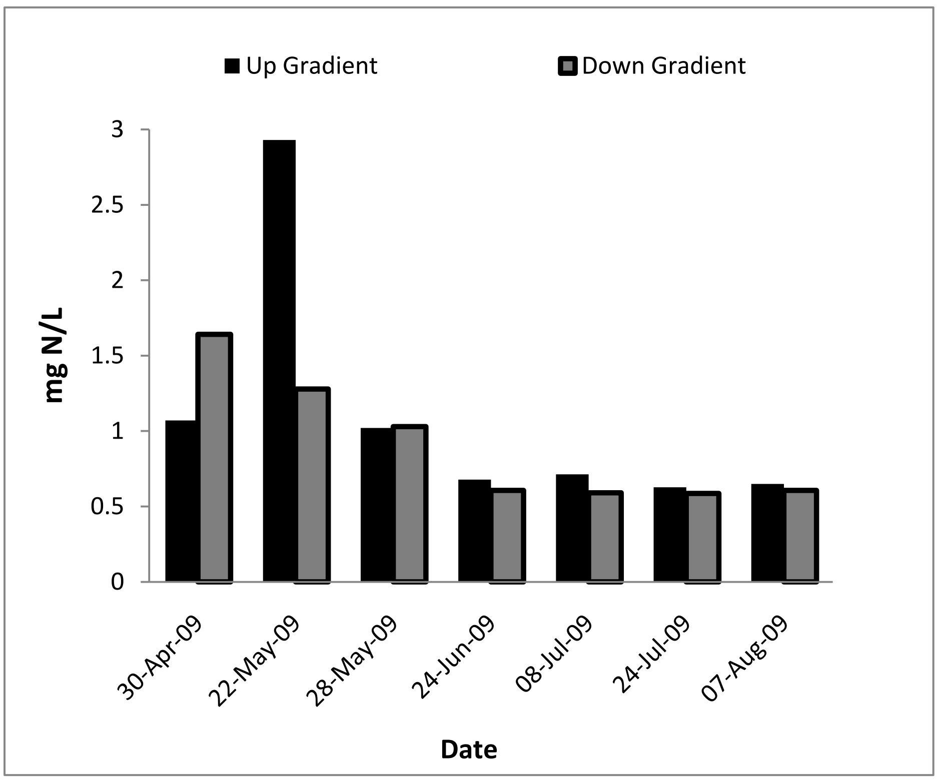

Figure 13. Groundwater TN values through time. Black bars represent groundwater samples taken up-gradient. Gray bars represent groundwater samples taken down-gradient. Values provided by MWTP.

Groundwater TP (Figure 14) represents the total concentration of all P forms, soluble and particulate. Up-gradient TP concentrations decreased throughout the growing season, from 0.21 mg/L on April 30 to 0.11mg/L on August 7, with the exception of one large value, 0.43 mg/L, recorded on May 28. Down-gradient TP concentrations were slightly more variable, ranging from 0.20 mg/L to 0.06 mg/L with no apparent pattern. It is important to note that TP values are higher at the start of our study than at the end.

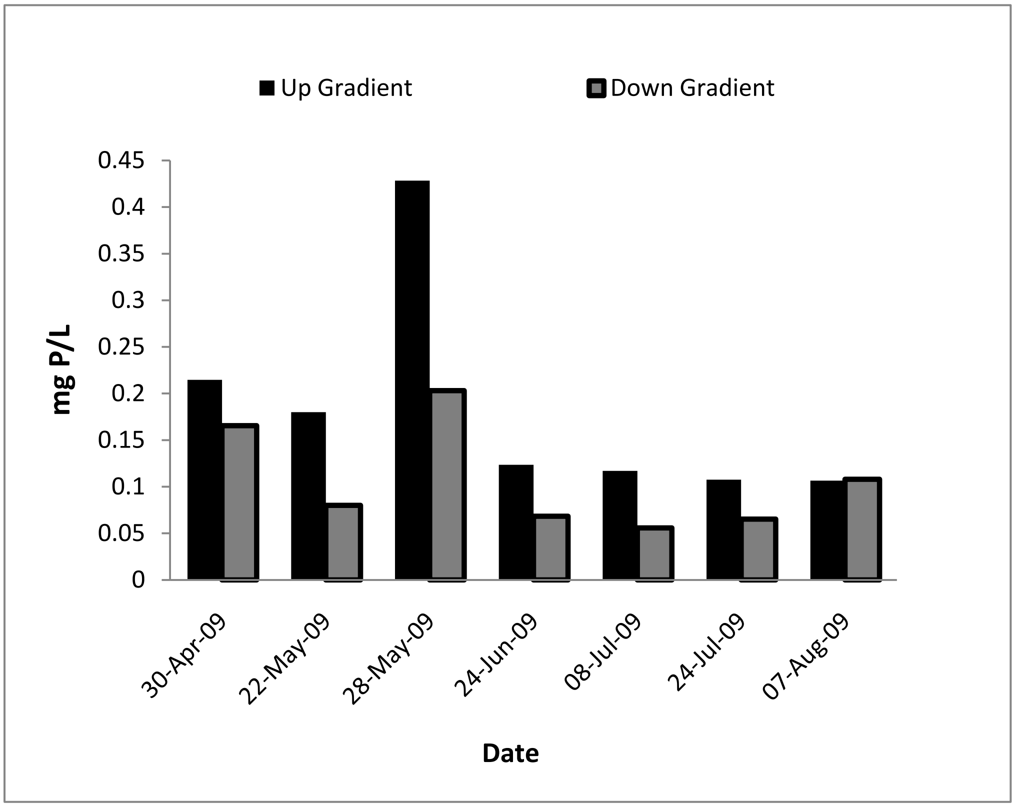

Figure 14. Groundwater TP values through time. Black bars represent groundwater samples taken up-gradient. Gray bars represent groundwater samples taken down-gradient. Values provided by MWTP.

Discussion

The primary objective of my study was to develop a Poplar plantation in Missoula, MT, which utilized effluent as a water and fertilization source. High survival rates indicate the development of a successful Poplar plantation in Missoula, MT, and the high relative growth rates of the effluent treated individuals (relative to the water-only individuals) indicates that fertilization had a strongly positive growth effect. The secondary objectives of my study were to access changes in soil and groundwater chemical properties resulting from effluent application. My results suggest that the effluent treatment had little – if any – effect on soil and groundwater chemistry.

Treated Effluent – Average effluent TN and TP values over the course of my study period were 8 ± 0.65 mg/L and 0.7 ± 0.12 mg/L respectively. A total of approximately 263,000 gallons (~ 1 million L) of effluent were irrigated on my 1.6 acre study plot in the first year. TN applied during our study was 8 ± 0.65 kg N while TP applied was only 0.7 ± 0.12 kg P. Compared to nutrient loads used in several recent studies (Moffat et al 2000, Cavaleri et al. 2004, Berthlot et al. 1999, Lteif et al. 2007, Barton et al. 2005, Brown and Driessche 2003), I applied smaller nutrient loads; however, these levels were purposely and appropriately conservative, given the lack of data and understanding concerning the use of Poplar plantations as a tertiary effluent treatment method.

Poplars – Clearly, the high Poplar survival rates and increased growth rates resulting from effluent irrigation suggest that poplar plantations may be a viable way to treat wastewater in this environment. Soil organic matter was greater in groundwater irrigation plots due to the remediation efforts by Rocking M. Design PC, which realistically means that these soils should have had more available nutrients than the highly degraded soils within the effluent irrigation plots at the start of this project. However, trees grown with effluent irrigation grew nearly twice as tall as those grown with groundwater alone. Given the condition of soils in the effluent watering area before treatment, this doubling affect in growth rates was likely driven, at least in part, by the fertilizing effect of the plant-available nutrients contained in the effluent irrigation.

Substantial amounts of data are readily available concerning Poplar survivorship and growth response to nutrient inputs. Each examined study reported increased Poplar growth resulting from fertilization (Felix et al. 2007, Fang et al. 2007, Cavaleri et al. 2004, Lteif et al. 2008, Easton and Petrovic 2004, Barton et al. 2005, Bungart and Huttl 2003, Deckmyn et al. 2003, Guillemettle and Des Rochers 2008, Moffat et al. 2000, Kelly and Ericsson 2002, Berthelot et al. 1999, Lteif et al. 2007, Brown and Driessche 2003). Of particular interest are reported increases in Poplar growth resulting from liquid organic fertilizers such as effluent. These studies also report that Poplar growth response is greater when given organic non-mineralized fertilizers compared to inorganic mineralized fertilizers (Moffat 2001, Lteif 2008, Cavaleri et al. 2004). Also of special interest is the success of Poplar plantations located on marginal or degraded soils. Felix et al. (2008) found a positive correlation between biomass accumulation and number of growth years for hybrid poplars located on a biosolid reentry site with low intensity (i.e., no additional fertilization, irrigation or pesticides usage), which was located on a former mining site. Lteif et al. (2007) not only found similar results for fertilized Poplars located on marginal agricultural lands, but also argued that the combination of fertilization and the presence of the Poplars led to improved soil nutrient quality and increased biological activity. I found very similar results on a degraded, highly disturbed site using three different Poplar species irrigated with treated effluent.

In recent years, the potential benefits and risks of growing hybridized poplars, especially in riparian zones occupied by native Poplars have been widely debated. Many hybridized Poplars – a number of which have been genetically modified in attempt to maximize growth rates – contain hidden defects in respect to diseases, insect resilience, and growth defects not yet clearly defined or understood (Dickmann 2001). Extremely high rate losses (e.g.98.5%) of native riparian zones have been recorded by some states (Braatne et al. 1996), raising concerns over the availability of a native gene bank for Poplars. Careful species selection must be applied when developing projects such as ours. I planted three varieties of Poplars in this study as an attempt to compare growth rates between native and hybrid species. With time, I should be able to assess the actual benefit, if any, of choosing a hybrid poplar over a native Cottonwood. At this point in time, however, the data do not suggest species specific differences in growth responses to effluent irrigation.

<u>Soils</u> – Soil TN decreased throughout the duration of our study, although I observed a small increase in the 0 to 30.5 cm depth interval from June to September, possibly due to organic matter introduction during weed management. This coincides with findings reported in a similar study by Kelly and Ericsson (2002), who also observed a decrease in soil nutrients overtime. The decrease observed in the Kelly and Ericsson (2002) study was attributed to plant uptake; however, I cannot confidently assess the mechanisms driving the TN decrease at my study site. TN decreases could be

the result of plant uptake, volatilization, or both. Future exploration of foliar nutrients could quantify Poplar nutrient uptake (St. John 2001, Moffat 2000, Cavaleri 2004, Berthelot 1999, Felix et al. 2007, Lteif 2008, Fang et al. 2007).

TP concentrations in the 0-30.5-cm depth interval decreased throughout the study period, whereas initial and final TP concentrations in the 30.5-61-cm depth were nearly equal. In a similar study, Berthelot et al. (1999) found TP levels greater at depth compared to shallow horizons, which coincides with our findings. Berthelot et al. (1999) suggested that seasonal declines in TP concentrations near the surface were due to plant uptake (Berthelot et al. 1999) whereas greater concentrations found at depth were a result of P immobilization and retention via geochemical sorption (Smil 2000). However, without a control plot with which to compare values, I cannot conclude that decreasing TP concentrations in the 0 to 30.5 cm depth were a result of plant nutrient uptake; likewise, I cannot conclude that an increase in concentration values at the 30.5 to 65 depth resulted from effluent watering alone. What I can conclude is that regardless of nutrient input from effluent irrigation, the soil strata showed little, if any, effects of effluent application.

Groundwater – Both groundwater TN and TP concentrations decreased throughout the study, with the exception of a spike in TN that occurred on 22 May 2009 and TP on 28 May 2009. Both of these increases were likely the result of an accidental over-watering of approximately 54,000 gallons of effluent on May 7 – 8. This over-watering event amounted to 22% of the allotted irrigation and occurred before Poplar root growth had begun; thus, nutrient retention from this event may have been dominated by microbial transformation, mineralization and retention via geochemical sorption, but not due to plant uptake. This event was perhaps one of the most interesting occurrences in my study as it allowed for the assessment of our groundwater monitoring well placement (Figure 7), while indicating the length of response time from irrigation event to groundwater impact. Response time for TN from effluent irrigation to groundwater interception could not be accurately assessed as groundwater samples were not taken between May 8 and May 22 (Figure 13). It is interesting to note that lower-gradient TN concentrations in samples from the May 22 sampling event were unaffected by the prolonged effluent irrigation event. Possible causes could be that the wells did not capture the effects I had anticipated due to possible groundwater channel changes that may have occurred between 1988 and 2009; or, the lack of an increase in groundwater N could simply reflect rapid N transformations from one form to another, or rapid N immobilization on cation exchange sites in the soil during infiltration (Kinzig and Socolow 1994, Vitousek 1997, Galloway 1998, Galloway 2003).

Both upper and lower-gradient groundwater TP concentrations during May 28 appear to be doubled, which again seems to be a result of the overwatering event. The doubling effect in both wells possibly confirms the findings of Woessner (1988) indicating that groundwater in the vicinity of our study plot flows West to South. Furthermore, the overwatering event informs our understanding of the temporal dynamics of P movement from effluent, through soils, and into groundwater. For example, the overwatering event occurred on May 7 – 8, but TP concentrations in groundwater were not detectable until the May 28 sample event. This would suggest a transit time of approximately 17 ± 4 days for P. Further sampling and analysis would be necessary to statistically assess this response time. Nonetheless, at the conclusion of the first growing season, the effluent irrigation rate used (~2041 gallons/day) appear to have had no impacts to ground water, whereas events of approximately 54,000 gallons/day appeared to drive increases in groundwater P concentrations. It is important to note, however, that there are currently no regulations describing maximum allowable TP in groundwater; whereas, TN levels have been limited to 10 mg/L. Throughout this study, groundwater TN levels remained below 3 mg/L, suggesting that effluent irrigation had very little, if any, effect on groundwater chemical composition.

Limitations – It is important to note that this study had some limitations. Perhaps most important, the experiment lacked a true control, which limited my ability to make statistically-based conclusions about the effects of effluent irrigation on soil chemistry and tree growth response. I had anticipated using the groundwater irrigation area (Figure 6) as a control plot for this experiment; however, the soils in this area were augmented with organic matter in 2004 by Rocking M. Designs, PC. Initial soil characteristics between the two plots were therefore very dissimilar. Had I more influence over the initial set-up of this project, I would have utilized a randomized block design that included plots within the treatment area which did not receive irrigation or trees. Utilization of this design would have allowed for the statistical rigor necessary to make definitive conclusions about the relative effects of effluent versus groundwater irrigation on tree growth and changes in soil chemistry. Given the current design, I cannot claim statistically significant effects of effluent application on soil and groundwater chemistry.

Another limitation of this project was that the study was developed with multiple competing objectives. The land application permit granted to the City of Missoula from the MTDEQ was the first permit of its kind in Montana and as a result contained strict loading regulations, loosely defined sampling techniques and required sampling frequency. As a result, a design utilizing only one type of hybrid Poplar was proposed by Morrison Maierles, Inc of Missoula. However, the proposed design restricted my ability to establish a scientifically rigorous project. In addition, initial site conditions were poor, land area was limited, and irrigation estimates were outdated (Appendix 3). Given these limitations, it is noteworthy that I was still able to establish a viable Poplar plantation, and that my results strongly suggest that the effluent had minimal collateral effects on soil and groundwater chemistry.

The real value of this study is that it does provide a blueprint for small communities (with small volumes of wastewater discharge) to follow if considering the implementation of a similar treatment system. Comparatively speaking, the recent BNR treatment upgrade in cost the City of Missoula approximately 18 million dollars, while my entire study and the treatment of 280,000 of effluent cost approximately $25,000. With more land area and more careful consideration of the plantation design projects of this nature could provide a valuable and cost-effective way of reducing small point source pollution inputs to surface waters during the growing season – while at the same time, utilizing nutrients to grow trees, or biomass, rather than unintentionally fertilizing harmful aquatic algae that could have profound negative effects on increasingly fragile water bodies.

Conclusion

Forty percent of US waterways are too polluted for basic uses such as swimming or fishing. The second largest contributor to surface water pollution is municipal wastewater treatment facilities. I set out to test an alternative tertiary wastewater treatment method that utilized Poplar trees irrigated with treated effluent. High Poplar survival rates and limited chemical affects to soil and groundwater chemical properties appear to justify this project as a success. Further research and investment in alternative wastewater treatment is necessary for the restoration and continued protection of our valuable and vulnerable water resources. This project provides a cost-effective and potentially effective alternative to municipal wastewater treatment approaches.

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Appendix A: Summary of Outfall 001 Discharge Monitoring Data.

Summary of Outfall 001 Discharge Monitoring Data

Parameter (mg/L unless noted)		Number of Samples	Average Value	Minimum Value	Maximum Value	Average Load (lb/day)
pH, s.u.	Annual	74	7.30	7.02	7.54	NA
	Winter [1]	54	7.12	6.34	7.21	NA
	Summer [2]	18	7.22	7.18	7.25	NA
Temperature, °C	Annual	74	15.0	10.1	19.8	NA
	Winter [1]	54	12.6	10.1	17.1	NA
	Summer [2]	18	18.5	16.6	19.8	NA
$cBOD_5$		74	4.3	1.4	12.5	291.8
TSS		74	7.4	2.3	16.9	506.6
Fecal Coliform Bacteria #/100ml, geometric mean		11	145	13	949	NA
Total Ammonia as N [3]	Annual	13	1.16	0.08	5.15	NA
	Winter [1]	9	0.99	0.16	5.15	NA
	Summer [2]	4	1.56	0.08	4.22	NA
Total Nitrogen as N [3]	Annual	13	9.27	6.34	10.80	635
	Winter [1]	9	9.82	10.84	7.11	691
	Summer [2]	4	8.02	9.35	6.34	549
Total Phosphorus as P [3]	Annual	13	0.81	0.12	1.62	55.8
	Winter [1]	9	0.98	0.58	1.62	69.2
	Summer [2]	4	0.43	0.12	0.78	29.6
Arsenic, Total Recoverable		24	0.001	<0.001	0.001	NA
Cadmium, Total Recoverable		24	0.0004	<0.0001	0.001	NA
Chromium, Total Recoverable		24	0.0012	<0.001	0.002	NA
Copper, Total Recoverable		24	0.0092	0.004	0.026	NA
Lead, Total Recoverable		24	0.0014	<0.001	0.002	NA
Mercury, Total Recoverable		24	<0.0004	<0.0001	<0.001	NA
Molybdenum, Total Recoverable		24	0.0052	<0.005	0.013	NA
Nickel, Total Recoverable		24	0.0039	<0.002	<0.005	NA
Selenium, Total Recoverable		24	0.001	<0.001	0.001	NA
Silver, Total Recoverable		24	0.0008	<0.0001	0.002	NA
Zinc, Total Recoverable		24	0.0437	<0.01	0.07	NA
Cyanide, Total		5	<0.005	<0.005	<0.005	NA

NA = Not Applicable/Not Available

1. Winter period is taken to be October 31 through May 31, annually.
2. Summer period is taken to be June 1 through September 30, annually.
3. Reduced data set is reflective of recent upgrade to BNR treatment at MWTP.

Appendix B: Current TMDL values for the Clark Fork River.

Enclosure

APPROVED TMDLS

Waterbody Name*	TMDL Pollutant	Water Quality Targets	TMDL (all values given as a 30 day averaging period and are based on the critical 30Q10 summer low flow condition): Location	WLA (kg/day)	LA** (kg/day)	TMDL (kg/day)
Clark Fork River USGS HUC 17010204 segments: MT76G001-1, MT76G001-2, MT76G001-3, MT76G001-4 USGS HUC 17010201 segments: MT76M001-1, MT76M001-2, MT76M001-3)	nitrogen (total) phosphorus (total)	**algae**: 100 mg/m^2 (summer mean) chlorophyll *a* 150 mg/m^2 (peak) chlorophyll *a* (at all locations in TMDL segments) **phosphorus**: 20 ug/l total phosphorus upstream of the Reserve Street bridge at Missoula 39 ug/l total phosphorus downstream of the Reserve Street bridge at Missoula **nitrogen:** 300 ug/l total nitrogen at all locations in TMDL segments **nutrient ratio:** 15:1 N:P ratio above Reserve Street bridge at Missoula	Silver Bow Creek ab. Butte		75 (TN), 2.7 (TP)	
			Butte WWTP	44 (TN), 4.4 (TP)		
			Clark Fork above Deer Lodge		52 (TN), 0.84 (TP)	
			Deer Lodge WWTP	0 (TN), 0 (TP)		
			Clark Fork below Deer Lodge			52 (TN), 0.84 (TP)
			Blackfoot River		184 (TN), 7.9 (TP)	
			Clark Fork above Missoula		285 (TN), 19 (TP)	
			Missoula	404 (TN), 40 (TP)		
			Clark Fork below Missoula			689 (TN), 59 (TP)
			Bitterroot River		414 (TN), 28 (TP)	
			Clark Fork above Stone Container		771 (TN), 54 (TP)	
			Stone Container (seepage) (direct)	30 (TN), 23 (TP) 0 (TN, 0 (TP)		
			Clark Fork Below Stone Container			801 (TN), 77 (TP)

* These waterbodies are currently on or have been on the State's Section 303(d) waterbody list. The TMDLs associated with these waters are considered Section 303(d)(1) TMDLs.
** Some of the Load Allocations include all upstream sources of the pollutant.
These TMDL, LA, and WLA values on this page are based on a 30 day average during summer months.

Appendix C: An examination of MTDEQ irrigation water requirement [IWR]

$$\text{IWR} = \frac{[Cu-(PPT_e+Soil\ Moisture)+\ LR]}{E_i}$$

Where: IWR = Irrigation water requirement
Cu = Crop consumptive use (in/acre/growing season)
PPT_e = Effective precipitation (in/growing season)
Soil Moisure = Assumed zero (in)
LR = Leaching requirement (%); assumed zero
E_i = Irrigation efficiency (%)

The IWR equation, developed by the Idaho DEQ, was adopted for this project by the MTDEQ. As LR and soil moisture are assumed zero, PPT_e, Cu, and E_i are the most important aspects of this equation. PPT_e, effective precipitation, refers to precipitation that remains in the soil and is available for plant uptake, and is generally estimated to be 70% of the measured growing season precipitation (MTDEQ 2006). MTDEQ PPT_e was estimated from the Western Regional Climate Center [WRCC], which utilizes data from the Missoula Airport. PPT_e for our site was estimated by calculating 70% of 7.86 inches per growing season, which yielded a value of 5.5 inches per growing season (MTDEQ 2006).

MTDEQ based Cu, crop consumptive use, on St. John's (2001) "Technical Notes: Hybrid Poplar An Alternative Crop for the Intermountain West." St John (2001), who reported first year water consumption by Poplars to be approximately 10 to 14 inches, simply restated data presented by James et al. (1989). In addition, there was a contractual agreement made between Washington State and Washington State University to update James' guidelines in 2008. This agreement concluded that James' irrigation outlines use old, out-dated estimation techniques and is not easy for the general public to access and use (WSDE 2008). In their defense, MTDEQ does state, and I concur, that limited research has been conducted on growth season water use by Poplars (MTDEQ 2006). However, presently available and easy to access research does indicate that Poplar's high photosynthesis rates are linked to high water demand (Deckmyn et al. 2003). Furthermore, it has been reported that the Poplar's acclaimed fast growth is strongly correlated to high levels of water (Hall 1997).

Irrigation efficiency [E_i] is the most powerful aspect of the IWR equation, larger values result in smaller IWR, whereas smaller values result in larger IWR. In this case, MTDEQ estimated E_i on Agrimet data from Corvallis, MT; an area located 45 miles south of Missoula. It should be noted that Corvallis data include evapotranspiration values for alfalfa, pasture land, lawns and spring grains; however, there are no data for trees, let alone high-water-use Poplars. The value chosen to estimate E_i in my study plot was 70%. The IWR final equation then becomes:

$$\text{IWR} = \frac{10-5.5}{.7} = 6.4 \text{ inches per acre} \sim 280{,}000 \text{ gallons/ year}$$

As noted in the body of this paper, this limitation was reached on July 25 and irrigation was continued with groundwater. Unfortunately, present data appear to suggest I could have continued watering with effluent and had little to no effect to soil and groundwater chemical properties.

Another compelling aspect to this argument is the nutrient loading limitations set forth by MTDEQ, which utilized estimates provided by Dr. Jon Johnson of Washington

State University Hybrid Poplar Research Program. Dr. Johnson estimated that first year Poplars would take up approximately 80 lb/acre/year N and 12 lb/acre/year P. Under the recommendation of Idaho DEQ, MTDEQ calculated an estimated 150% N and 125% P load allocation based on Dr. Johnson's estimates, resulting in loading limitations of 120 lb/acre/year N and 15 lb/acre/year P. However, when calculating the total delivered nutrients in the IWR of 280,000 gallons/year of effluent containing concentrations of 10 mg/L TN and 1 mg/L TP, the resulting maximum yearly nutrient loads are only 23 lb/year TN and 2 lb/year TP. These limitations seem incredibly conservative when considering the big picture – I am attempting to create a system that utilizes treated effluent, ordinarily discharged into the Clark Fork River, as an irrigation source for Poplars; a species known for its rapid nutrient and water uptake qualities.

It would seem that more accurate, area specific IWRs and nutrient loads could be formulated for projects such as ours. At the very least, allocations to the IWR should be considered and readily available in order to adjust watering allotments in an event such as the overwatering occurrence on 7 and 8 May of this study.